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Abstract

This study utilized DNA fingerprint probes and a progeny performance test to identify genetic markers linked to quantitative trait loci. The BC_1-sires and their F_2-progeny originate from line cross of two genetically distinct egg-type lines of chickens. Multiple DNA fingerprint bands of the sires were found to be associated with daughter performance in growth, reproduction and egg quality. About 25% of QTL-associated bands previously identified by gradient analysis were confirmed by progeny tests, and many additional genetic markers were identified that had not been detected by gradient analysis.

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GENETIC MARKERS LINKED WITH QUANTITATIVE TRAITS IN CHICKENS

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SUMMARY

This study utilized DNA fingerprint probes and a progeny performance test to identify genetic markers linked to quantitative trait loci. The BC_1-sires and their F_2-progeny originate from line cross of two genetically distinct egg-type lines of chickens. Multiple DNA fingerprint bands of the sires were found to be associated with daughter performance in growth, reproduction and egg quality. About 25% of QTL-associated bands previously identified by gradient analysis were confirmed by progeny tests, and many additional genetic markers were identified that had not been detected by gradient analysis.

INTRODUCTION

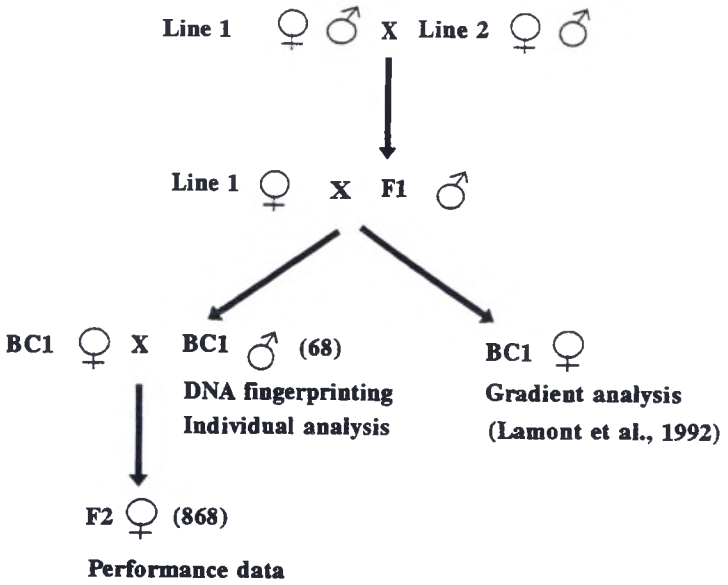
Genetic markers associated with quantitative trait loci (QTL) are of great value in breeding programs, particularly for traits which are sex-limited, expressed late in life or measurable only after slaughter. Due to their dispersed genomic location, variable number tandem repeat (VNTR, Nakamura et al., 1987) sequences used to probe animal genomes to identify QTL-associated DNA fingerprint bands have facilitated identification of genetic markers (Jeffreys et al., 1985; Vassart et al., 1987; Longmire et al., 1990; Haberfeld and Hillel, 1991 and Benkel and Gavora, 1993). DNA fingerprint bands linked to QTLs can be used as genetic markers to aid classical genetic selection for improving quantitative traits of economic importance (Dunnington et al., 1992). In poultry, and other animals, possible linkages between quantitative traits and DNA fingerprint bands have been reported (Georges et al., 1990; Dunnington et al., 1990 and Plotsky et al., 1993). Methods that pool individual samples, such as tail analysis (Dunnington et al., 1992) and gradient analysis (Lamont et al., 1992) have been used for initial screening for genetic markers. The simplicity and economy associated with these techniques make them attractive approaches to screen for DNA fingerprint bands potentially associated with QTL, but these analyses will not prove linkage between genetic markers and QTLs nor provide accurate estimates of band effect. Previously, we used VNTR probes and gradient analysis on BC_1-hens for the initial screening of potential genetic markers of QTLs (Lamont et al., 1992). The second phase of this study, reported here, used BC_1-male DNA for individual VNTR fingerprinting to identify genetic markers associated with QTLs based on the quantitative trait performance of their F_2-female progeny.

MATERIALS AND METHODS

The linkage disequilibrium in the experimental population was introduced by crossing two genetically distinct, but phenotypically similar, commercial egg-type lines (Lines 1 and 2, Figure 1). Sixty-eight BC_1-sires from the above cross were used for individual DNA fingerprinting analysis. Quantitative trait performance data (body weight, egg weight, shell quality, shell color, albumen height, sexual maturity (age at first egg), percent rate of lay after first egg, total eggs laid) were collected from F_2-female progeny sired by the 68 BC_1 males. Genomic DNA used for DNA fingerprinting was isolated from blood samples (Dunnington et al., 1990). DNA isolated from blood pools (15 to 18 birds) from Line 1 and Line 2 were used as among-gel standards. Eight μg of HinfI digested DNA was size separated in agarose gels and transferred to nylon membranes.

Two VNTR probes, 33.6 (Jeffreys et al., 1985) and R18.1 (Haberfeld and Hillel, 1991) were used to identify DNA fingerprint bands. Membranes were prehybridized for 1-2 hours at 65°C with 30 ml of buffer and hybridized overnight at 65°C after the addition of 25ng of ^{32}P-dCTP labelled probe. Membranes were washed once with 0.263M Na_2HPO_4, 1.0% SDS; twice with 2X SSC, 0.1% SDS; and twice with 1X SSC and 0.1% SDS. All washes were at 65°C for 20 minutes. Membranes were exposed to X-ray films at room temperature for 1-2 days for autoradiography. DNA fingerprint patterns were visually scored for band presence.

Figure 1. Experimental population used in this study



The quantitative trait performance data collected from 868 F_2-hens were adjusted for the hatch means and deviations from the hatch mean for each trait were used in the statistical analyses. The data were analyzed by General Linear Model (GLM) procedure (SAS Inc., Cary, NC) for sire band effect on progeny performance by grouping F_2-hens by sire status (presence or absence of each DNA fingerprint band).

RESULTS

Fifty-two DNA fingerprint bands (27 with probe R18.1; 25 with probe 33.6), ranging from 2 to 23 Kbp in size, were analyzed for QTL marker identification with the 8 traits, for a total of 416 band-by-trait analyses. A total of 101 (49 for probe R18.1 and 52 for probe 33.6) associations of DNA markers and quantitative traits, or approximately 25%, were significant ($P \leq 0.05$). The number of DNA fingerprint bands associated with each trait ranged from 2 to 8 for probe R18.1 and 2 to 9 for probe 33.6 (Table 1). The range of least squares estimations of band effects on deviation from the hatch means for each trait are listed in Table 1.

Table 1. Number of genetic markers associated with quantitative traits and range of estimated sire band effects on F_2 progeny performance.

Trait	QTL marker		Estimated loss/gain[1]	
	R18.1	33.6	R18.1	33.6
Body weight(kg)	3	9	-0.09 to 0.08	-0.04 to 0.12
Egg weight(gm)	9	2	-1.42 to 1.92	-1.10 to 1.26
Shell quality[2](2-oz)	5	9	-0.67 to 0.55	-2.09 to 0.52
Albumen height(mm)	8	8	-0.34 to 0.35	-0.24 to 0.67
Sexual maturity(day)	8	7	-4.79 to 3.15	-11.55 to 7.41
Egg production[3](%)	2	3	-2.51 to-1.91	-2.39 to 2.30
Total eggs[4]	7	6	-5.81 to 2.65	-6.08 to12.33
Shell color[5]	7	8	-1.68 to 1.34	-4.40 to 0.59

[1] Least squares mean of the deviation from the hatch mean ($P< 0.05$). Range represents most extreme effects of bands significantly associated with each trait
[2] Resistance to puncture breakage
[3] Percent rate of lay from date of sexual maturity to 267 days
[4] Total number of eggs laid from sexual maturity to 267 days
[5] Reflectance units ranging from 15(white) to 100(black)

DISCUSSION

Selection of breeders based on phenotypic values of the individual or its relatives is not always a good indicator of genotype, especially for quantitative traits with low heritability.

DNA analysis using multilocus probes has proven to be a useful tool in identifying markers associated with QTL in poultry (Dunnington et al., 1992; Dolf et al., 1993; Plotsky et al., 1993). In this study, we identified DNA fingerprint bands linked with quantitative traits of economic importance based on the relationship of F_2-progeny performance and the BC_1-sires' DNA fingerprint patterns. Of the 62 markers previously found by gradient analysis to be associated with performance in BC_1-hens(Lamont et al., 1992), only about 25% (15 of 62) were confirmed with the present individual DNA typing and progeny testing. This is in agreement with the proportion of markers detected by tail analysis screening which were confirmed by progeny test in the study of Dunnington et al. (1992). The individual sire fingerprinting and progeny tests, however, revealed 86 additional genetic markers of QTLs, not previously detected by gradient analysis. This study illustrates the independent value of each type of strategy for identifying genetic markers of QTLs. Production of locus- or allele-specific probes for these candidate markers can facilitate marker-assisted selection in poultry breeding. Chromosome walking on the chromosomal segments localized by genetic markers will enhance the ability to identify and clone the actual QTLs.

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